

Nanomedicine: By Small Means

An Honors Thesis (HONRS 499)

By

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Eric R. Hedin, 12/4/07

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Abstract

Nanomedicine is fundamentally interdisciplinary and complex. Therefore, it requires utmost expertise in chemistry, biology, physics, and engineering. With rapid strides being made in nanotechnology, the field of nanomedicine is coming into being. As it is very new, it is a revolutionary time in the field of science. However, complications arise. First, because the field is so complex, methods, approaches, and goals vary immensely and it becomes enormously difficult to make sense of it.

The purpose of this project is to provide a course outline for a survey course which could be taught at the undergraduate or graduate level to explain what the field is, what is being done, and how it is being done, as well as supplying some interdisciplinary training which was most likely not included in their previous training. In addition, a presentation which describes the course and the field of nanomedicine itself has been prepared and will be given at multiple venues, including the Butler University Undergraduate Research Conference in Spring 2008.

An essay, in addition to the original aims of the project, has been included. This essay, titled "Nanomedicine: an Introduction" serves as an introduction to this hypothetical textbook.

Acknowledgements

-I want to thank Dr. Eric Hedin for his continuous support and guidance throughout this project. His invaluable help was an integral part of the process.

-I also want to thank Linley and Mark Baker for the tremendous amount of support they provided on many levels during the project and from multiple continents.

-I also want to thank Dr. Ryan Torrie and for his guidance and recommendation of this field of study.

Daniel Baker

Dr. Eric Hedin

Honors Senior Project

Nanomedicine: By Small Means

Introduction

Section I.: Tools and Methods of Research

Chapter 1: Fabrication

Chapter 2: Imaging and Analysis

I. Microscopy

II. Spectroscopy

III. Chemical Analysis

Chapter 3: Computer Modeling

I. Ab Initio Methods

II. Numerical Analysis

III. Monte Carlo

IV. Methods of Approximation

Chapter 4: Interdisciplinary Studies

I. Biochemistry

II. Molecular Biology

1. Emulation of Biological Systems

2. Biomolecule Utility

III. Biophysics

IV. Inorganic Chemistry

1. Complexation

Section II.: Nanoparticles

Chapter 5: Properties

Chapter 6: Varieties

Chapter 7: Fabrication

Chapter 8: Industrial Applications

Section III.: Nanomedicine

Chapter 9: Targeting

I. Tracing

II. Delivery

Chapter 10: Biosensors

I. Chemical Regulation

Chapter 11: Disease Control

Chapter 12: Oncology

Chapter 13: Cell Repair

Chapter 14: Systems Applications

I. Neuroelectronic Interfaces

Chapter 15: Nanomachines and Nanorobots

Epilogue

Chapter 16: Bioethics

I. Data Protection

II. Intellectual Property

III. Legal Concerns

List of Pertinent Abstracts

Each topic is given a tag to the outline. Each tag is given a 3-digit code which will facilitate searching for papers on subject.

Tags:

Imaging **1

Modeling **2

Fabrication **3

Interdisciplinary Studies **4

Biomolecules as Tools **5

Nanoparticles **6

Industrial Applications **7

Medical Applications:

Biosensors **8

Targeting **9

Oncology *10

Disease Control *11

Nanomachines *12

Repair *13

Systems applications *14

Ethics *15

Abstracts, organized by name, hyperlinked by topic, from Åkerman to Zhao.

Åkerman, et al.

Nanocrystal targeting *in vivo*

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Contributed by Erkki Ruoslahti, August 1, 2002

Inorganic nanostructures that interface with biological systems have recently attracted widespread interest in biology and medicine. Nanoparticles are thought to have potential as novel intravascular probes for both diagnostic (e.g., imaging) and therapeutic purposes (e.g., drug delivery). Critical issues for successful nanoparticle delivery include the ability

to target specific tissues and cell types and escape from the biological particulate filter known as the reticuloendothelial system. We set out to explore the feasibility of *in vivo* targeting by using semiconductor quantum dots (qdots). Qdots are small (<10 nm) inorganic nanocrystals that possess unique luminescent properties; their fluorescence emission is stable and tuned by varying the particle size or composition. We show that ZnS-capped CdSe qdots coated with a lung-targeting peptide accumulate in the lungs of mice after i.v. injection, whereas two other peptides specifically direct qdots to blood vessels or lymphatic vessels in tumors. We also show that adding polyethylene glycol to the qdot coating prevents nonselective accumulation of qdots in reticuloendothelial tissues. These results encourage the construction of more complex nanostructures with capabilities such as disease sensing and drug delivery.

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[Full Text of Åkerman et al.] [Reprint (PDF) Version of Åkerman et al.]

Targeting **9, Nanoparticles **6, Biosensors **8

Benenson, et al.

DNA molecule provides a computing machine with both data and fuel

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Edited by Peter B. Dervan, California Institute of Technology, Pasadena, CA, and approved January 13, 2003 (received for review September 17, 2002)

The unique properties of DNA make it a fundamental building block in the fields of supramolecular chemistry, **nanotechnology**, nano-circuits, molecular switches, molecular devices, and molecular computing. In our recently introduced autonomous molecular automaton, DNA molecules serve as input, output, and software, and the hardware consists of DNA restriction and ligation enzymes using ATP as fuel. In addition to information, DNA stores energy, available on hybridization of complementary strands or

hydrolysis of its phosphodiester backbone. Here we show that a single DNA molecule can provide both the input data and all of the necessary fuel for a molecular automaton. Each computational step of the automaton consists of a reversible software molecule/input molecule hybridization followed by an irreversible software-directed cleavage of the input molecule, which drives the computation forward by increasing entropy and releasing heat. The cleavage uses a hitherto unknown capability of the restriction enzyme *FokI*, which serves as the hardware, to operate on a noncovalent software/input hybrid. In the previous automaton, software/input ligation consumed one software molecule and two ATP molecules per step. As ligation is not performed in this automaton, a fixed amount of software and hardware molecules can, in principle, process any input molecule of any length without external energy supply. Our experiments demonstrate 3×10^{12} automata per μl performing 6.6×10^{10} transitions per second per μl with transition fidelity of 99.9%, dissipating about 5×10^{-9} W/ μl as heat at ambient temperature.

[†]Y.B. and R.A. contributed equally to this work.

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[Full Text of Benenson et al.] [Reprint (PDF) Version of Benenson et al.]

Industry Application- electronics, computing. **7 , Biomolecules as Tools **5

Cherukuri, et al.

Mammalian pharmacokinetics of carbon nanotubes using intrinsic near-infrared fluorescence

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Communicated by Robert F. Curl, Rice University, Houston, TX, October 20, 2006
(received for review July 31, 2006)

Individualized, chemically pristine single-walled carbon nanotubes have been intravenously administered to rabbits and monitored through their characteristic near-infrared fluorescence. Spectra indicated that blood proteins displaced the nanotube coating of synthetic surfactant molecules within seconds. The nanotube concentration in the blood serum decreased exponentially with a half-life of 1.0 ± 0.1 h. No adverse effects from low-level nanotube exposure could be detected from behavior or pathological examination. At 24 h after i.v. administration, significant concentrations of nanotubes were found only in the liver. These results demonstrate that debundled single-walled carbon nanotubes are high-contrast near-infrared fluorophores that can be sensitively and selectively tracked in mammalian tissues using optical methods. In addition, the absence of acute toxicity and promising circulation persistence suggest the potential of carbon nanotubes in future pharmaceutical applications.

nanoparticle biodistribution | nanoparticle toxicity | luminescence spectroscopy | single-walled carbon nanotubes

Freely available online through the PNAS open access option.

[†]Deceased October 28, 2005.

Author contributions: P.C., H.K.S., R.E.S., S.A.C., and R.B.W. designed research; P.C., C.J.G., and T.K.L. performed research; P.C., C.J.G., T.K.L., S.A.C., and R.B.W. analyzed data; and R.B.W. wrote the paper.

Conflict of interest statement: R.B.W. holds an interest in Applied NanoFluorescence, LLC.

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[Full Text of Cherukuri et al.] [Reprint (PDF) Version of Cherukuri et al.]

Nanoparticles **6, Targeting **9, Tracing **9

Ellis-Behnke, et al.

**Nano neuro knitting: Peptide nanofiber scaffold for
brain repair and axon regeneration with
functional return of vision**

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Communicated by D. Carleton Gajdusek, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France, January 23, 2006 (received for review November 9, 2005)

Nanotechnology is often associated with materials fabrication, microelectronics, and microfluidics. Until now, the use of nanotechnology and molecular self assembly in biomedicine to repair injured brain structures has not been explored. To achieve axonal regeneration after injury in the CNS, several formidable barriers must be overcome, such as scar tissue formation after tissue injury, gaps in nervous tissue formed during phagocytosis of dying cells after injury, and the failure of many adult neurons to initiate axonal extension. Using the mammalian visual system as a model, we report that a designed self-assembling peptide nanofiber scaffold creates a permissive environment for axons not only to regenerate through the site of an acute injury but also to knit the brain tissue together. In experiments using a severed optic tract in the hamster, we show that regenerated axons reconnect to target tissues with sufficient density to promote functional return of vision, as evidenced by visually elicited orienting behavior. The peptide nanofiber scaffold not only represents a previously undiscovered nanobiomedical technology for tissue repair and restoration but also raises the possibility of effective treatment of CNS and other tissue or organ trauma.

CNS regeneration | tissue repair | nanomedicine

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Author contributions: R.G.E.-B., K.-F.S., and G.E.S. designed research; R.G.E.-B., Y.-X.L., S.-W.Y., and G.E.S. performed research; S.Z. contributed new reagents/analytic tools; R.G.E.-B., Y.-X.L., D.K.C.T., K.-F.S., and G.E.S. analyzed data; R.G.E.-B., K.-F.S., and G.E.S. wrote the paper; and K.-F.S. and G.E.S. provided financial support.

** Schneider, G. E., You, S., So, K. F., Carter, D. A., Khan, F., Okobi, A. & Ellis-Behnke, R. (2000) *Soc. Neurosci. Abstr.* **26**, 611.

Conflict of interest statement: No conflicts declared.

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Systems applications *14 , Repair *13 , Biomolecules as Tools **5 , Molecular Biology **4 , Nanoparticles **6

Helmke, Brian P., and Adrienne R. Minerick.

2.Designing a nano-interface in a microfluidic chip to probe living cells: Challenges and perspectives

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Edited by Peter G. Wolynes, University of California at San Diego, La Jolla, CA, and approved March 23, 2006 (received for review August 22, 2005)

Nanotechnology-based materials are beginning to emerge as promising platforms for biomedical analysis, but measurement and control at the cell–chip interface remain challenging. This idea served as the basis for discussion in a focus group at the recent National Academies Keck *Futures Initiative*. In this Perspective, we first outline recent advances and limitations in measuring nanoscale mechanical, biochemical, and electrical interactions at the interface between biomaterials and living cells. Second, we present emerging experimental and conceptual platforms for probing living cells with nanotechnology-based tools in a microfluidic chip. Finally, we explore future directions and critical needs for engineering the cell–chip

interface to create an integrated system capable of high-resolution analysis and control of cellular physiology.

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[Full Text of Helmke and Minerick] [Reprint (PDF) Version of Helmke and Minerick]

Biosensors **8 , Molecular Biology **4 , Biomolecules as Tools **5 , Chemical Regulation *12

Hyman, et al.

Design of protein struts for self-assembling nanoconstructs

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Edited by Jack Halpern, University of Chicago, Chicago, IL, and approved April 29, 2002 (received for review October 12, 2001)

Bacteriophage T4 tail fibers have a quaternary structure of bent rigid rods, 3×160 nm in size. The four proteins which make up these organelles are able to self-assemble in an essentially irreversible manner. To use the self-assembly domains of these proteins as elements in construction of mesoscale structures, we must be able to rearrange these domains without affecting the self-assembly properties and add internal binding sites for other functional elements. Here we present results on several alterations of the P37 component of the T4 tail fiber that change its length and add novel protein sequences into the protein. One of these sequences is an antibody binding site that is used to inactivate phage carrying the modified gene.

Nanomachines *12, Fabrication **3, Molecular Biology **4, Biomolecules as Tools **5

LaVan, et al.

Approaches for biological and biomimetic energy conversion

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Edited by Peter G. Wolynes, University of California at San Diego, La Jolla, CA, and
approved February 23, 2006 (received for review August 4, 2005)

This article highlights areas of research at the interface of nanotechnology, the physical sciences, and biology that are related to energy conversion: specifically, those related to photovoltaic applications. Although much ongoing work is seeking to understand basic processes of photosynthesis and chemical conversion, such as light harvesting, electron transfer, and ion transport, application of this knowledge to the development of fully synthetic and/or hybrid devices is still in its infancy. To develop systems that produce energy in an efficient manner, it is important both to understand the biological mechanisms of energy flow for optimization of primary structure and to appreciate the roles of architecture and assembly. Whether devices are completely synthetic and mimic biological processes or devices use natural biomolecules, much of the research for future power systems will happen at the intersection of disciplines.

biotechnology | nanotechnology | photosynthesis | photovoltaic

Author contributions: D.A.L. and J.N.C. wrote the paper.

[¶] The National Academies Keck Futures Initiative, Designing Nanostructures Conference, November 19–21, 2004, Irvine, CA.

^{||} Birge, R. R., Chen, Z., Govender, D. S. K., Stuart, J. A., Tallent, J. R. & Tan, E. H. L., 212th ACS National Meeting, Aug. 25–29, 1996, Orlando, FL, p. PHYS-123.

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Osakada, et al.

Charge transfer through DNA nanoscaled assembly programmable with DNA building blocks

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Edited by Paul F. Barbara, University of Texas, Austin, TX, and approved October 3, 2006

DNA nanostructures based on programmable DNA molecular recognition have been developed, but the nanoelectronics of using DNA is still challenging. A more rapid charge-transfer (CT) process through the DNA nanoassembly is required for further development of programmable DNA nanoelectronics. In this article, we present direct absorption measurements of the long-range CT over a 140-Å DNA assembly based on a GC repetitive sequence constructed by simply mixing DNA building blocks. We show that a CT through DNA nanoscale assembly is possible and programmable with the designed DNA sequence.

transient absorption measurement | nanostructure hole transfer | DNA oxidation | nanotechnology

Author contributions: Y.O., K.K., M.F., and T.M. designed research, performed research, contributed new reagents/analytic tools, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

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[Full Text of Osakada et al.] [Reprint (PDF) Version of Osakada et al.]

Pan, Bifeng, et al.

Dendrimer-Modified Magnetic Nanoparticles Enhance Efficiency of Gene Delivery System

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Magnetic **nanoparticles** (MNP) with a diameter of 8 nm were modified with different generations of polyamidoamine (PAMAM) dendrimers and mixed with antisense *survivin* oligodeoxynucleotide (asODN). The MNP then formed asODN-dendrimer-MNP composites, which we incubated with human tumor cell lines such as human breast cancer MCF-7, MDA-MB-435, and liver cancer HepG2 and then analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, quantitative reverse transcription-PCR, Western blotting, laser confocal microscopy, and high-resolution transmission electron microscopy. Results showed that the asODN-dendrimer-MNP composites were successfully synthesized, can enter into tumor cells within 15 min, caused marked down-regulation of the *survivin* gene and protein, and inhibited cell growth in dose- and time-dependent means. No.5 generation of asODN-dendrimer-MNP composites exhibits the highest efficiency for cellular transfection and inhibition. These results show that PAMAM dendrimer-modified MNPs may be a good gene delivery system and have potential applications in cancer therapy and molecular imaging diagnosis. [Cancer Res 2007;67(17):8156–63]

Nanoparticles **6 , Imaging **1 , Molecular Biology **4 , Oncology *10, Targeting **9

Roy, et al.

Optical tracking of organically modified silica nanoparticles as DNA carriers: A nonviral, nanomedicine approach for gene delivery

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Communicated by Peter M. Rentzepis, University of California, Irvine, CA, October 28, 2004 (received for review June 10, 2004)

This article reports a multidisciplinary approach to produce fluorescently labeled organically modified silica nanoparticles as a nonviral vector for gene delivery and biophotonics methods to optically monitor intracellular trafficking and gene transfection. Highly monodispersed, stable aqueous suspensions of organically modified silica nanoparticles, encapsulating fluorescent dyes and surface functionalized by cationic-amino groups, are produced by micellar nanochemistry. Gel-electrophoresis studies reveal that the particles efficiently complex with DNA and protect it from enzymatic digestion of DNase 1. The electrostatic binding of DNA onto the surface of the nanoparticles, due to positively charged amino groups, is also shown by intercalating an appropriate dye into the DNA and observing the Förster (fluorescence) resonance energy transfer between the dye (energy donor) intercalated in DNA on the surface of nanoparticles and a second dye (energy acceptor) inside the nanoparticles. Imaging by fluorescence confocal microscopy shows that cells efficiently take up the nanoparticles *in vitro* in the cytoplasm, and the nanoparticles deliver DNA to the nucleus. The use of plasmid encoding enhanced GFP allowed us to demonstrate the process of gene transfection in cultured cells. Our work shows that the nanomedicine approach, with nanoparticles acting as a drug-delivery platform combining multiple optical and other types of probes, provides a promising direction for targeted therapy with enhanced efficacy as well as for real-time monitoring of drug action.

nonviral vector | ORMOSIL nanoparticles | confocal microscopy

Author contributions: I.R., T.Y.O., D.J.B., H.E.P., and P.N.P. designed research; I.R., T.Y.O., D.J.B., H.E.P., R.A.M., and N.K. performed research; I.R., T.Y.O., D.J.B., H.E.P., N.K., and P.N.P. analyzed data; and I.R., T.Y.O., D.J.B., H.E.P., and P.N.P. wrote the paper.

Abbreviations: ORMOSIL, organically modified silica; VTES, triethoxyvinylsilane; APTES, 3-aminopropyltriethoxysilane; Aerosol-OT, dioctyl sodium sulfosuccinate; FRET, fluorescence resonance energy transfer; Rh6G, rhodamine 6G; HPPH, 2-devinyl-

2-(1-hexyloxyethyl)pyropheophorbide; CT, calf thymus; EMA, ethidium monoazide bromide; EthD-1, ethidium homodimer-1; pEGFP, plasmid encoding EGFP.

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[Full Text of Roy et al.] [Reprint (PDF) Version of Roy et al.]

Molecular Biology **4, Biomolecules as Tools **5, Nanoparticles **6, Targeting **9

Sanders, Charles R.

Visiting order on membrane proteins by using nanotechnology

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Molecular Biology **4 , Biomolecules as Tools **5 , Imaging **1 .

Seeman, Nadrian C., Angela M. Belcher.

Emulating biology: Building nanostructures from the bottom up

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The biological approach to nanotechnology has produced self-assembled objects, arrays and devices; likewise, it has achieved the recognition of inorganic systems and the control of their growth. Can these approaches now be integrated to produce useful systems?

[Full Text of Seeman and Belcher] [Reprint (PDF) Version of Seeman and Belcher]

Molecular Biology **4 , Biomolecules as Tools **5 , Industry Application-electronics, computing. **7

Souza, et al.

Networks of gold nanoparticles and bacteriophage as biological sensors and cell-targeting agents

Glauco R. Souza^{*}, Dawn R. Christianson^{*}, Fernanda I. Staquicini^{*}, Michael G. Ozawa^{*}, Evan Y. Snyder[†], Richard L. Sidman^{‡,§}, J. Houston Miller[¶], Wadih Arap^{*,§}, and Renata Pasqualini^{*,§}

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Contributed by Richard L. Sidman, November 10, 2005

Biological molecular assemblies are excellent models for the development of nanoengineered systems with desirable biomedical properties. Here we report an approach for fabrication of spontaneous, biologically active molecular networks consisting of bacteriophage (phage) directly assembled with gold (Au) nanoparticles (termed Au-phage). We show that when the phage are engineered so that each phage particle displays a peptide, such networks preserve the cell surface receptor binding and internalization attributes of the displayed peptide. The spontaneous organization of these targeted networks can be manipulated further by incorporation of imidazole (Au-phage-imid), which induces changes in fractal structure and near-infrared optical properties. The networks can be used as labels for enhanced fluorescence and dark-field microscopy, surface-enhanced Raman scattering detection, and near-infrared photon-to-heat conversion. Together, the physical and biological features within these targeted networks offer convenient multifunctional integration within a single entity with potential for **nanotechnology**-based biomedical applications.

target | fractal | hydrogel | stem cell | assembly

Author contributions: G.R.S., D.R.C., F.I.S., M.G.O., R.L.S., J.H.M., W.A., and R.P. designed research; G.R.S., D.R.C., F.I.S., and M.G.O. performed research; E.Y.S., R.L.S., J.H.M., W.A., and R.P. contributed new reagents/analytic tools; G.R.S., D.R.C., F.I.S., M.G.O., J.H.M., W.A., and R.P. analyzed data; and G.R.S., D.R.C., F.I.S., M.G.O., R.L.S., J.H.M., W.A., and R.P. wrote the paper.

Conflict of interest statement: No conflicts declared.

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Abbreviations: imid, imidazole; NIR, near infrared; SERS, surface-enhanced Raman scattering; TEM, transmission electron microscopy; TU, transducing units; Df, fractal dimension.

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[Full Text of Souza et al.] [Reprint (PDF) Version of Souza et al.]

Nanoparticles **6, Fabrication **3, Molecular Biology **4, Biomolecules as Tools **5, Targeting **9, Biosensors **8

Zhao, et al.

A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles

Xiaojun Zhao^{*}, Lisa R. Hilliard^{*}, Shelly John Mechery^{*}, Yanping Wang[†], Rahul P. Bagwe^{*}, Shouguang Jin[†], and Weihong Tan^{*,†}

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Edited by Nicholas J. Turro, Columbia University, New York, NY, and approved September 9, 2004 (received for review July 5, 2004)

The rapid and sensitive determination of pathogenic bacteria is extremely important in biotechnology, medical diagnosis, and the current fight against bioterrorism. Current methods either lack ultrasensitivity or take a long time for analysis. Here, we report a bioconjugated nanoparticle-based bioassay for *in situ* pathogen quantification down to

single bacterium within 20 min. The bioconjugated nanoparticle provides an extremely high fluorescent signal for bioanalysis and can be easily incorporated with biorecognition molecules, such as antibody. The antibody-conjugated nanoparticles can readily and specifically identify a variety of bacterium, such as *Escherichia coli* O157:H7, through antibody–antigen interaction and recognition. The single-bacterium-detection capability within 20 min has been confirmed by the plate-counting method and realized by using two independent optical techniques. The two detection methods correlated extremely well. Furthermore, we were able to detect multiple bacterial samples with high throughput by using a 384-well microplate format. To show the usefulness of this assay, we have accurately detected 1–400 *E. coli* O157 bacterial cells in spiked ground beef samples. Our results demonstrate the potential for a broad application of bioconjugated nanoparticles in practical biotechnological and medical applications in various biodetection systems. The ultimate power of integrating **bionanotechnology** into complex biological systems will emerge as a revolutionary tool for ultrasensitive detection of disease markers and infectious agents.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: RuBpy, Tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate; CFU, colonyforming units.

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[Full Text of Zhao et al.] [Reprint (PDF) Version of Zhao et al.]

Fabrication **3, Molecular Biology **4, Biomolecules as Tools **5, Nanoparticles **6, Imaging **1, Modeling **2

Daniel Baker

Honors Senior Project

Dr. Eric Hedin

Nanomedicine: an Introduction

Introduction

Nanotechnology is a new phenomenon which is attempting to push the boundaries of all fields of science to their quantum limits. Computing, imaging, electronics, medicine, and many other fields are being revolutionized. The motivation is clear: better, quicker, smaller, and more efficient devices are of great value and nanoscience claims the power to produce these results.

There are three general methodologies for pursuing a nanomedicine technology. In order of difficulty and value, they are nanoscale materials technology, biotechnology (or a *wet* nanomedicine), and molecular nanotechnology (*dry*). Freitas, who, at the time of writing, is in the process of finishing his three-book series on the field, has provided a table describing the differences in medical challenges alone. As one can see, efficiency and efficacy increase along with the difficulty. I am of the opinion that these fields need to be developed in sequence, "Gradus Ad Parnassum." Not only will this be more realistic, but it will provide benefits in the near future, giving us further incentive to develop. It will take time to realize our potential.

Table 1-4 Medical Challenges of Increasing Difficulty and Possible Approaches

Level of Difficulty	Specific Medical Challenge	Increasing Efficacy →		
		Homeostatic or Nanomaterials Technology	Biototechnology Approach	Molecular Nanotechnology Approach
I	Minor Symptoms and Minor Physical Trauma	Herbal preparation, minor surgery, passage of time	Symptomatic suppressors pharmacology	Pharmacovs, zippocytes, respicytes, etc.
II	Infectious Agents	Natural immune system	Antibiotics and vaccines	Nanobiotics
III	Mutation and Cell Disease	Natural immune system Major surgery	Molecular diagnostics and treatments, gene therapies	Nanobiotics Cell repair machines
IV	Health Maintenance and Aging	Vitamins, good diet, exercise, moderation, and "Yi Jien Jing"	Gene therapy to enhance self-repair and function function	Whole-body cyto assay Cell repair machines
V	Major Organ Replacement or Repair	Organ transplantation Major surgery	Stem cell & tissue engineering embryonic gene reactivation	Cell milks, tissue milks organ milks, nanosurgery
VI	Morpho-Engineering	Cosmetic surgery	Control of natural morphogenetic systems	Nanoburgery Chromatin editors
VII	Emergency Care: Restoration of Nonhomeostatic Tissue	Emergency major surgery Prosthesis or bionics	Biological repair, nets Bioengineered immune cells	Traumatopods Warm biostases
VIII	Augmentation of Natural Structure or Function	Prosthesis or bionics	Cross-species and artificial morphogenetic supplementation	Cell engineering devices Autogenous control

Freitas, Robert A., Jr. Example 1.

I. Nanoscale Materials Technology

Nanoscale materials technology is already in limited use today. It is defined as technology using materials whose size ranges from 0.1nm to 100 nm (Freitas). These materials lack autonomy after fabrication. Its applications include targeting, tracing, and industrial applications. Industrial applications range from sunscreen to bacteria-resistant paint. Essentially, nanoscale materials technology is the application of nanofabrication to specific problems. Nanoparticles, biomolecules, and other objects created with nanoscale precision are the tools. Its limitations are that it can only solve specific problems and is less refined than the other techniques of which we will speak.

“Nanoscale materials technology has already found widespread use in medicine, including biocompatible materials and analytical techniques, surgical and dental practice, nerve cell research using intracellular electrodes, biostructures research and biomolecular research using near-field optical microscopy, scanning-probe microscopy and optical tweezers, and vaccine design, and also many 20th century bulk chemical and biochemical manufacturing techniques along with much of classical pharmacology”

(Freitas)

Genes have been targeted, inhibited, and even delivered, without the need for either of our two more complicated nanobrother fields (Pan, et al.), (Roy, et al.). Essentially, this gives us control, allowing us to implant genes we want and turn off genes we don't want. This highly specific genetic control could have tremendous possibilities. For example, genes whose expression leads to cancer and other diseases can be eliminated.

Nanoparticles, fairly easily produced, can be used to deliver drugs to specific places in the body and not where it isn't needed, offering improved efficiency over conventional drugs. Similarly, it provides detailed information about where drugs and other chemicals go in the body when they are put into the system. Thermal therapy with nanoshells and chemotherapy with nanoparticles designed to conjugate with specific cancer cells are two emerging oncological tools. Because gold nanoparticles essentially do nothing to the system, they are safe carriers for chemicals and effectively deliver the drug to the cancer cells and not to the cells of the host organism.

Drugs can be rapidly “bioassayed,” or tested for effect on living organisms.

(Zhao, et al.) This will radically change pharmaceutical industry and the methodology of research. “The ultimate power of integrating bionanotechnology into complex biological systems will emerge as a revolutionary tool for ultrasensitive detection of disease markers and infectious agents” (Zhao, et al.)

Artificial tissues and organs can be grown with control of antigens, genetic code, differentiation, and function, leading possibly to organs. Rehabilitation will be made possible in ways that have been hopeless.

Examples

Author: **Ellis-Behnke, et al.**

Title: Nano-neuroknitting: Peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision

Description: Amine chains fabricated can repair neurological systems and bring back sight.

Author: **Helmke, Brian P., and Adrienne R. Minerick**

Title: Designing a nano-interface in a microfluidic chip to probe living cells

Description: A chip-cell interface would allow measuring of mechanical, chemical, and biological interactions and greatly increase the efficiency of data collection.

Author: **Zhao, et al.**

Title: A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles

Description: Nanoparticles complexed with test molecules can be quickly tested in sequence for function.

Author: **Roy, et al.**

Title: Optical tracking of organically modified silica nanoparticles as DNA carriers:
A nonviral, nanomedicine approach for gene delivery

Description: Silica was complexed with amine groups and dyes that were tracked to deliver genes *in vitro*.

*Table 1.3. Comparison of Macroscale and Biomolecular Components and Functions*¹⁸²

Macroscale Device	Device Function	Biomolecular Examples
Struts, beams, casings	Transmit force, hold positions	Microtubules, cellulose
Cables	Transmit tension	Collagen
Fasteners, glue	Connect parts	Intermolecular forces
Solenoids, actuators	Move things	Conformation-changing proteins, actin/myosin
Motors	Turn shafts	Flagellar motor
Drive shafts	Transmit torque	Bacterial flagella
Bearings	Support moving parts	Sigma bonds
Containers	Hold fluids	Vesicles
Pumps	Move fluids	Flagella, membrane proteins
Conveyor belts	Move components	RNA moved by fixed ribosome (partial analog)
Clamps	Hold workpieces	Enzymatic binding sites
Tools	Modify workpieces	Metallic complexes, functional groups
Production lines	Construct devices	Enzyme systems, ribosomes
Numerical control systems	Store and read programs	Genetic system

Freitas, Robert A., Jr. Example 2.

II. Biotechnology

Biotechnology in living systems has a complete system of nanomachines and devices, mass-produced at the molecular level, serving as a model for molecular nanotechnology as well as a system in itself. Biotechnology is defined as the use of

— living organisms and biological techniques. There are two approaches to this study: the first is an emulation of biological systems to create a Molecular Nanotechnology, and the other is a manipulation of existing biological machines, robots, and molecules for a literal Biotechnology.

Biotechnological systems can be very effective, as evidenced by all life. An organism is immensely complicated from every perspective, but existing systems work with exceptionally high redundancy nonetheless; they are designed to be tolerant of the many other processes that are going on. If an error takes place, the body makes the necessary adjustments.

For a simple example, the body keeps its pH between 7.1 and 7.3. If the pH goes outside that range, the body breaks down. The use of buffer solutions in the body, along with other mechanisms, keeps the body in that small range.

— This characteristic is precisely what makes biotechnology so attractive: it is self-regulating and self-reproducing. A system which possesses these attributes would require little outside maintenance and would therefore facilitate practicality for widespread implementation. Existing cells or organelles could be modified to perform given tasks. Freitas has proposed a method for transforming a white blood cell, or leukocyte, into a nanorobot capable of control of biosystem control, along with a necessary experimental information required.

In short, biotechnology could be reliable, but bulky and slow. Its advantages over nanoscale materials technology are redundancy, resilience, and sustainability. Its advantage over Molecular Nanotechnology is that it would require only a modification of existing nanomachines instead of the invention of them from the bottom up.

Examples

Author: **Benenson, et al.**

Title: DNA molecule provides a computing machine with both data and fuel

Author: **LaVan, et al.**

Description: DNA is an excellent candidate for nanocomputer because it can be powered by ATP and it stores its own information already.

Title: Approaches for biological and biomimetic energy conversion

Author: **Oskada, et al.**

Title: Charge transfer through DNA nanoscaled assembly programmable with DNA building blocks

Description: DNA's unique properties modify its conductance capabilities. These are explored in ways applied to computing.

Author: **Souza, et al.**

Title: Networks of gold nanoparticles and bacteriophage as biological sensors and cell-targeting agents

Description:

III. Molecular Nanotechnology

Molecular nanotechnology is, essentially, engineering effective machines at the nanoscale. The key difference between Biotechnology and Molecular Nanotechnology is

a bottom-up perspective. Freitas cites 14 advantages of Molecular Nanotechnology over Biotechnology. The main benefits are speed, power density, building materials, control, versatility and reliability. Because biological systems are made for their precise purpose, they have high specificity to their tasks and therefore are limited. However, several researchers, such as Oskada and Benenson are attempting to overcome this limitation. They do precisely what they were made to do. Engineers want to work with something versatile and capable of performing a variety of functions and molecular nanotechnology is the only field which will be able to fulfill all those requirements.

One major method for planning this technology is emulation of biological systems. Since they provide analogs for so many macroscale devices, it provides excellent examples.

Being “bottom-up,” it will be more involved. We will need to create analogs of macroscopic machines, such as generators, engines, and devices for locomotion, energy storage and metabolism, and computing. There are, therefore, far fewer examples in this field.

Example

Author: **Seeman, Nadrian C., Angela M. Belcher.**

Title: Emulating biology: Building nanostructures from the bottom up

Description: Seeman uses biological systems as analogs for a molecular nanotechnology.

Author: **Freitas, Robert A., Jr.**

Title: Exploratory Design in Medical Nanotechnology: A Mechanical Artificial Red Cell

Description: Freitas proposes methods by which a an analog to an RBC could be made to function by mechanical processes.

Conclusion

In summary, these three fields each offer unique benefits attached to their own costs. All three should therefore be pursued. Depending on one's field, they will affect not only personal health but what one's job as a scientist is. The nano-paradigm will replace the micro- and macro-paradigms. Their focus of research would be very different.

Basic and clinical research will be fundamentally different in subject, method, and practice. Medical practice will have more rapid and effective diagnosis and treatment. Finally, in the end, life expectancy and quality will potentially be dramatically improved and elongated. Disease, infirmity, disability and injury may be prevented and treated with unprecedented speed and effectiveness. If these become a reality, nanomedicine could be become the most important development in Western medicine.

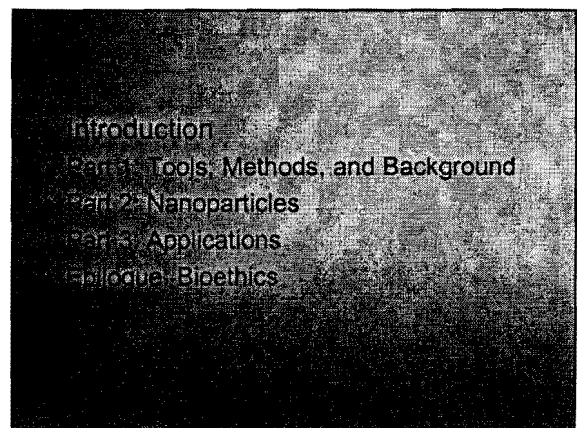
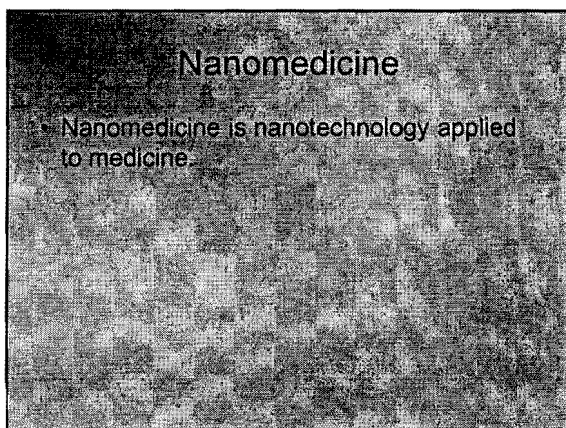
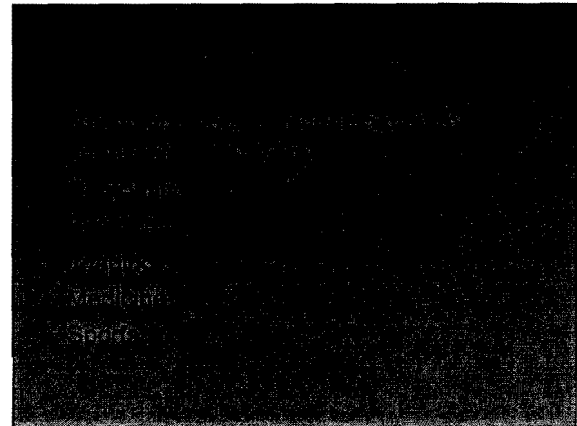
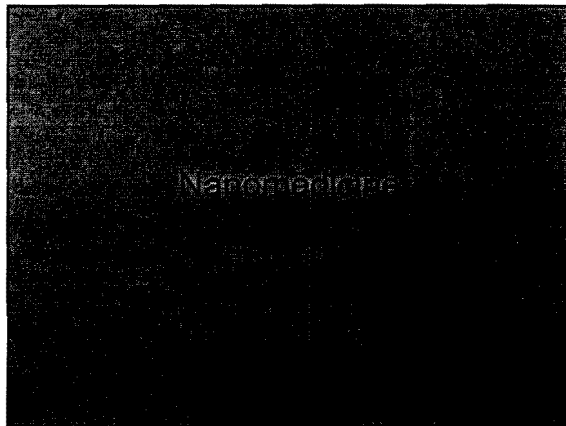
All that remains is to expand the initiative to realize these possibilities, and now is the time to start. The door is open. Whole new fields are created with increasing frequency with more focused specialties. It seems that there's still "plenty of room at the bottom." The time has come to grab a metaphorical shovel and start digging. Good luck.

References

- Benenson, et al. "DNA molecule provides a computing machine with both data and fuel" *Proc Natl Acad Sci U S A*. 2003 Mar 4;100(5):2191-6. Epub 2003 Feb 24.
- Ellis-Behnke, et al. "Nano-neuroknitting: Peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision" *Proc Natl Acad Sci U S A*. 2006 Mar 28;103(13):5054-9. Epub 2006 Mar 20.
- Freitas, Robert A., Jr., Nanomedicine, Vol. I: Basic Capabilities. Published online, 1998. Availability: <http://www.nanomedicine.com>.
- Freitas Robert A., Jr., "Exploratory Design in Medical Nanotechnology: A Mechanical Artificial Red Cell," *Artificial Cells, Blood Substitutes, and Immobil. Biotech.* **26**(1998):411-430.
- Helmke, Brian P., and Adrienne R. Minerick., "Designing a nano-interface in a microfluidic chip to probe living cells: Challenges and perspectives." *PNAS*, **103-17**(2006), 6419-6424.
- LaVan, et al. "Approaches for biological and biomimetic energy conversion" *PNAS*, **103-14**(2006). 5251-5255.
- Oskada, et al. "Charge transfer through DNA nanoscaled assembly programmable with DNA building blocks" *Proc Natl Acad Sci* **103(48)**: 18072–18076.
- Roy, et al. "Optical tracking of organically modified silica nanoparticles as DNA carriers: A nonviral, nanomedicine approach for gene delivery" *PNAS*, **102-2**(2005), 279-284.
- Seeman, Nadrian C., Angela M. Belcher. "Emulating biology: Building nanostructures from the bottom up" *PNAS*, 99(2002) ,Suppl. 2, 6451-6455.
- Seeman, et al. "New Motifs In DNA Nanotechnology". Availability: <http://www.foresight.org/Conferences/MNT05/Papers/Seeman/index.html>

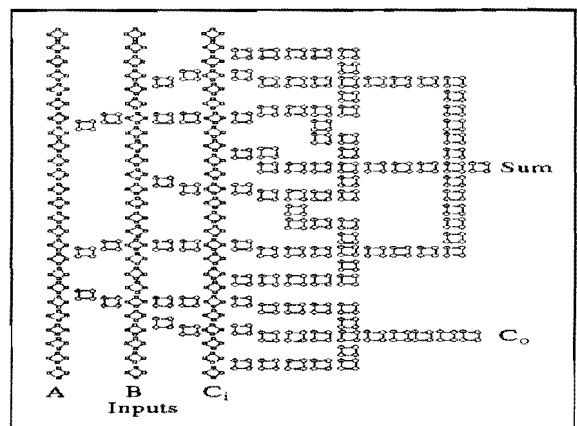
Souza, et al. "Networks of gold nanoparticles and bacteriophage as biological sensors and cell-targeting agents" *PNAS*, **103-5**(2006), 1215-1220.

Zhao, et al. "A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles" *Proc Natl Acad Sci U S A*. 2004 Oct 19;101(42):15027-32. Epub 2004 Oct 11.



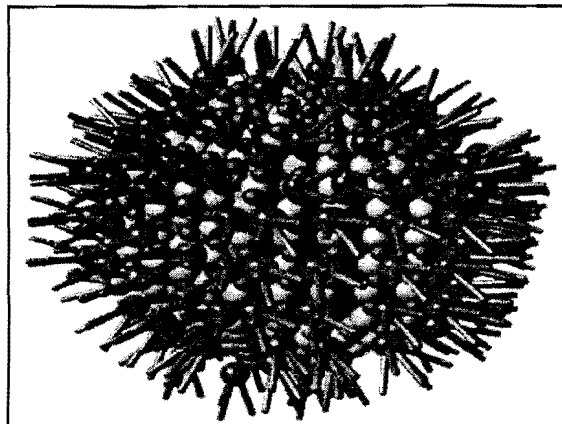
Computing

- QCA
 - Quantum Dot Cellular Automata
- Quantum Computing
- Optical Computing
 - Military Applications



Molecular Electronics

- DNA
 - Provides its own fuel, compact
 - Can function as both transistor and conductor
- Single-Molecular



The Anatomy of the NDMX Golfball

- Machined 432 dimples increase lift and distance
- High strength "Proprietary Metallic Core" provides structural base enabling high moments of inertia
- High performance polymer layer maximizes energy transfer between club face and steel core
- Tough urethane cover for durable performance

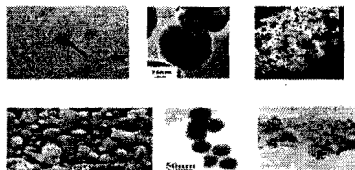


3 Main Methods

- Nanoscale Materials Technology
 - Sunscreen, Makeup
- Bionanotechnology
 - DNA as transistor
- Molecular Nanotechnology
 - Nanomachines
 - Nanorobots

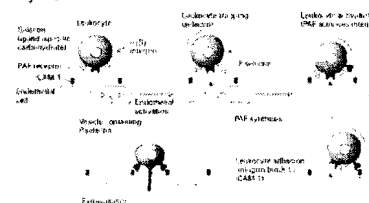
Nanoscale Materials

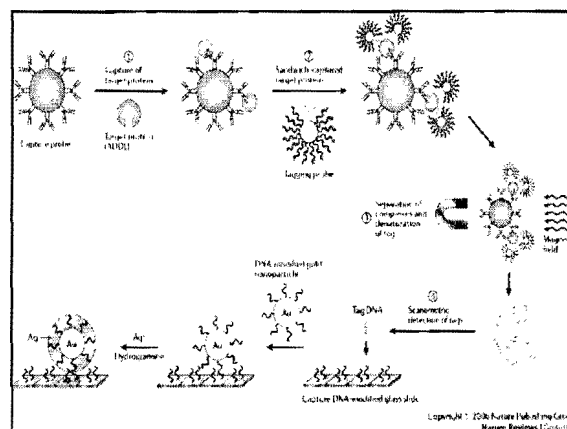
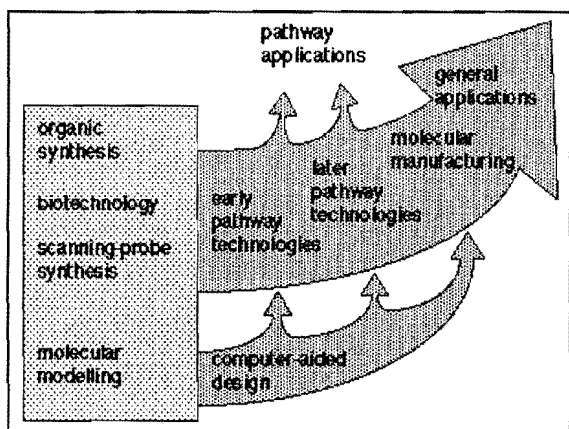
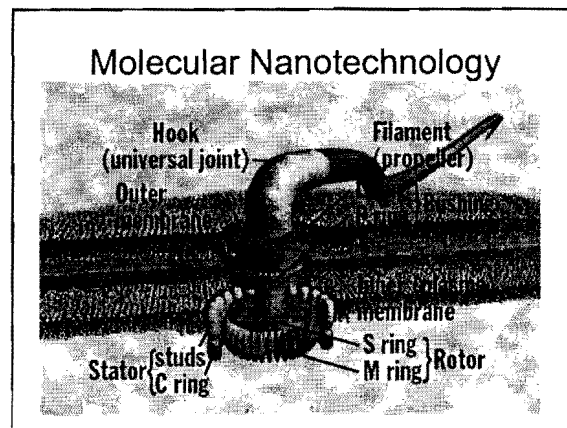
- Nanoparticles
- Biomolecules
- Other objects created with nanoscale precision are the tools.



Biotechnology

- Biotechnology has a complete system of nanomachines and devices, mass-produced at the molecular level, serving as a model for molecular nanotechnology as well as a system in itself.

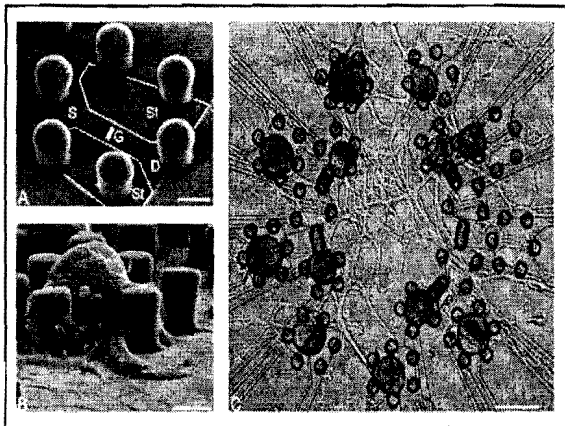
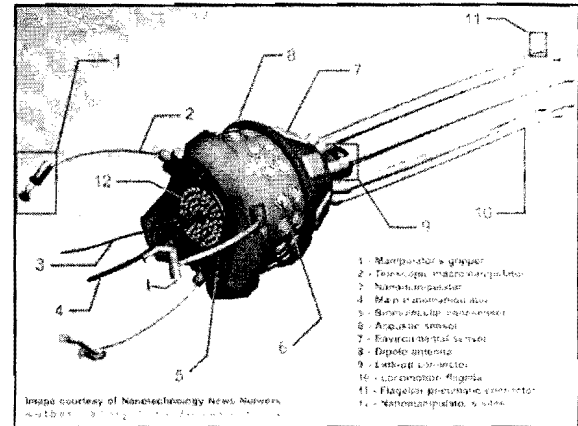
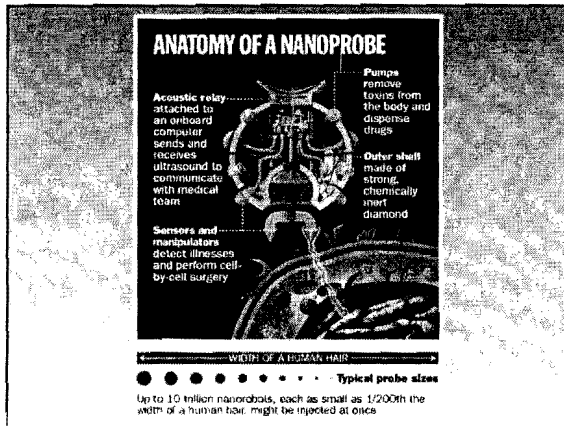




- Nanomedicine is fundamentally interdisciplinary→
- Fields
 - Chemistry
 - Physics
 - Biology

- Imaging Breast Cancer





Bioethics

- Intellectual Rights
- New Genetic Engineering Issues
- Data Protection/Encryption
- Unpredicted side effects

Conclusion

- There's Plenty of Room at the bottom.
- Nanoscale Materials are where we start.
- Biotechnology provides both inspiration for Molecular Nanotechnology and a valuable path itself.
- Molecular Nanotechnology has the greatest potential with the greatest costs.

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References

- Benenson, et al. "DNA molecule provides a computing machine with both data and fuel." *Proc Natl Acad Sci U S A* 2005 Mar 4;102(10):11518-11523. Epub 2005 Feb 24.
- Ellis-Behnke, et al. "Nanoneurotrophin: Peptide nanolipid scaffold for brain repair and axon regeneration with functional return of vision." *Proc Natl Acad Sci U S A* 2006 Mar 28;103(13):5354-9. Epub 2006 Mar 20.
- Freitas, Robert A. Jr. "Nanotechnology 3000 L.S.: Basic Nanobioethics." Published online, 1991. Availability: <http://www.nanoethics.com>
- Freitas Robert A. Jr. "Extraterrestrial Design for Medical Nanotechnology: A Mechanical Artificial Red Cell." *Artificial Cells, Blood Substitutes, and Immobilized Biotech*. 2011;39(4):411-430.
- Holmes, Brian P. and Adrienne R. Mirvick. "Designing a nanointerface in a microfluidic chip to probe living cells: Challenges and perspectives." *PNAS*, 103-10(2006): 6418-6424.
- LIVEN, et al. "Approaches for biological and biomimetic energy conversion." *PNAS*, 103-10(2006): 5251-5255.
- Lomonaco, Michael D. "And Will They Go Inside Us? Given the Promise of Nanotechnology, It's a Safe Bet." *Time Magazine* 154 (9 November 1999): 53.
- Oshroff, et al. "Charge transfer through DNA nanocatalytic assembly programmable with DNA building blocks." *Proc Natl Acad Sci* 102(48): 15673-15678.
- Roy, et al. "Optical tracking of organically modified silica nanoparticles as DNA carriers: A noninvasive, nonmedicative approach for gene delivery." *PNAS*, 103-2(2006): 375-384.
- Seeman, Nathan C., Angus M. Belcher. "Smulating biology: Building nanomaterials from the bottom up." *PNAS*, 100(2002): 8401-8406.
- Seeman, et al. "New Molecules in DNA Nanotechnology." Availability: <http://www.forsyth.org/Conferences/NHT05/papers/Seeman/index.html>
- Prasad, et al. "Nanorobots of gold nanoparticles and bacteriophage as biological sensors and cell-targeting agents." *PNAS*, 103-5(2006): 1215-1220.
- Zhao, et al. "A rapid bioassay for single bacterial cell quantitation using bioengineered nanoparticles." *Proc Natl Acad Sci U S A*, 2004 Oct 14;101(42):15027-32. Epub 2004 Oct 11.